Relation of Serum and Muscle Free Amino Acids to Dietary Protein in the Northern Bobwhite

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This study investigated the effects of dietary protein content on body condition and used serum and muscle free amino acids to assess the status of juvenile Northern Bobwhite quail (*Colinus virginianus*). Thirty-six juveniles were subjected to three experimental dietary protein treatments (8, 15, and 33% crude protein). Body mass was reduced in Bobwhites fed either 15 or 8% protein diets. Total concentrations of sulfur-containing amino acids, and ratios of branched-chain to non-branched chain amino acids, essential amino acid to nonessential amino acids, and glycine to (leucine + valine) in blood serum were useful for discriminating among diet groups. Likewise, concentrations of sulfur-containing amino acids and glycine were useful discriminators for muscle tissue homogenates. Profiles of selected amino acids in serum and muscle can be used for assessing protein nutritional status and diet quality of Bobwhites.

INTRODUCTION

Many useful techniques have been developed for assessing the overall nutritional status or health of Northern Bobwhite quail *(Colinus virginianus),* including measures of body mass, body composition, selected organ and glandular development, and parasite burden (*1*,*2*). The most frequent approach is measuring lipid reserves, such as total weight of dissectable or extractable body fat, gizzard fat stores, and fatty acid composition of selected depots of fat (*3*). Although these techniques are successful indices of chronic nutritional status, particularly with respect to energy intake, they do not easily detect short-term alterations in diet quality or animal condition (*4*). Techniques for assessing acute changes in protein nutritional status of Northern Bobwhites are limited.

Altered nutritional states in birds often yield rapid adjustments in physiological homeostasis, resulting in measurable changes in blood chemistry (*5*,*6*). Similar approaches have been used to assess alterations in physiology of wild Northern Bobwhites during the breeding season (*7*). The poorly defined sensitivity of most measures of physiological responses to alterations in diet quality limits their value in nutritional assessment, especially with regard to protein nutrition, in Northern Bobwhites.

Profiles of free amino acid concentrations in serum and muscle tissue have been used successfully to evaluate the protein nutritional status of laboratory animals (*8*) and humans (*4*). Concentrations of free amino acids in serum and tissues represent a balance between amino acid intake in food, rate of use in protein synthesis, and muscle catabolism or amino acid oxidation (*9*). As intake of protein, and to some degree energy, is modified, the physiological rates involved in maintenance of amino acid homeostasis also can be expected to change (*10*). Because protein malnutrition can lead to the differential metabolism of certain amino acids relative to others, many clinical evaluations of malnutrition also incorporate evaluations of specific ratios such as essential to nonessential, serine to threonine, and tyrosine to neutral amino acids (*11*).

We have been examining alternatives to the traditional morphological indices used to assess condition in Bobwhites. Current techniques that evaluate chronic energy status are of limited use in assessing acute changes in protein nutritional status. This study examines the sensitivity of free amino acid pools in serum and muscle tissue of juvenile Northern Bobwhites to three levels of protein in the diet under controlled experimental conditions. The effects of dietary protein on traditional gravimetric indices of condition are also presented. It was hypothesized that clinical evaluations incorporating concentrations of free amino acids in serum and muscle would provide a more sensitive assessment of nutritional status

than traditional morphological indices.

MATERIALS and METHODS

Animals and Experimental Design. Thirty-six juvenile Northern Bobwhites (4 weeks-old) were weighed, banded, and randomly assigned to one of three experimental diets varying in protein content (8, 15, or 33% crude protein) for a 4-week trial. Dietary protein levels were based on known requirements of wild and captive Northern Bobwhites (*12,13*). Birds were hatched from a captive stock and raised under a natural photoperiod in a battery brooder containing vertical decks (4×4×2 m), located in a well-ventilated housing facility approved for use by the Institutional Laboratory Animal Resources Committee at Oklahoma State University. Each experimental diet group was housed together in adjacent pens under identical environmental conditions. Temperature in the housing facility was maintained relatively constant (27.3 to 32.3 °C) by using heat lamps and regulating ventilation. Water and experimental diets were provided for *ad lib* consumption throughout the trial.

Experimental protein diets were made isocaloric by varying the concentration of starch (Table 1); crude protein content was determined by Kjeldahl analysis and amino acid composition for the three experimental diets was calculated (Table 1). Body mass was recorded at weekly intervals. Daily feed consumption of juvenile birds was measured by offering a known quantity of feed and weighing uneaten portions the next day.

Birds were returned to the laboratory after the trials, anesthetized with 5 mg ketamine hydrochloride (Aveco Co., Inc., Fort Dodge, IA 50501), and exsanguinated via the jugular vein at 0700 h. Blood samples were collected in vacuum tubes, allowed to clot, and centrifuged at 10^3 × g for 10 min at 15 °C. Serum was decanted and stored frozen at -20 °C for amino acid analysis.

TABLE 1. Ingredient composition (%) and calculated amino acid composition (percent air-dry basis) of isocaloric 8, 15, 33% protein diets fed to juvenile Northern Bobwhites.

Ingredient	8% CP	15% CP	33% CP
Starch	53.31	45.60	30.00
Ground corn	21.67	21.35	20.94
Soybean meal[a]	7.56	16.16	32.66
Animal fat	12.09	11.89	11.63
Dicalcium phosphate[b]	3.32	2.96	2.61
Vitamin mix[c]	0.45	0.44	0.44
Methionine (99%)	0.45	0.44	0.44
Salt (NaCl)	0.45	0.44	0.44
Limestone (38% Ca)	0.45	0.57	0.68
Trace mineral mix[d]	0.15	0.15	0.15
Essential amino acids			
Arginine	0.62	1.17	2.23
Histidine	0.25	0.43	0.80
Isoleucine	0.39	0.74	1.42
Leucine	0.84	1.42	2.54
Lysine	0.47	0.91	1.77
Methionine	0.66	0.84	1.20
Phenylalanine	0.47	0.85	1.57
Threonine	0.34	0.62	1.14
Tryptophan	0.09	0.19	0.39
Valine	0.48	0.83	1.52
Nonessential amino acids			
Aspartic acid	0.90	1.74	3.36
Cysteine	0.10	0.20	0.38
Glutamic acid	1.76	3.15	5.79
Glycine	0.34	0.64	1.21
Proline	0.52	0.89	1.59
Serine	0.48	0.86	1.58
Tyrosine	0.32	0.59	1.12

a 84.5% crude protein content; Nurish 3000, Protein Technologies International, St. Louis, MO 63164.
b 18.5% P, 22% Ca, 18% F; Pitman-Moore Inc.
c Per kilogram of premix: vitamin A (palmitate) 3,968,280 IU; cholecalciferol 1, 102,300 IU; vitamin E (dl–tocopherol), 13,228 IU; vitamin B12, 7.9 mg; riboflavin, 2,646 mg; niacin, 17,637 mg; d–pantothenic acid, 4,409 mg; choline, 200,178 mg; menadione, 728 mg; folic acid, 441 mg; pyridoxine, 1,587 mg; thiamine, 794 mg; d–biotin, 44 mg; Hoffmann-LaRoche Inc., Nutley, NJ 07110.
d Ca 15.00%, Zn 10.00%, Mn 12.00%, Fe 7.50%, Cu 1.00%, I 0.25%; J. M. Huber Corp., Quincy, IL 62301.

Body Condition Analysis. Post-mortem examination included determination of the masses of the whole body, liver, gizzard, gizzard fat, spleen, adrenal glands, and reproductive organs. Carcasses were dried by lyophilization and ground to a fine powder with a food processor and microgrinding mill. Body fat was assessed by ether extraction with a Soxhlet apparatus (*14*) and ash content was determined by combustion in a muffle furnace at 600 °C for 6 h. Percent body protein was determined by subtracting percentages of fat, water, and ash from 100 (*15*).

Amino Acid Analysis. Fifty milligrams of breast muscle tissue was homogenized in 300 1 of 0.1N HCl and centrifuged at $1.5\times10^3\times$g for 25 min. Serum and muscle homogenate from each bird was deproteinized by filtering through a 10^4 molecular weight cut-off ultrafiltration membrane filter

(Ultrafree-MC, Millipore, Bedford, MA 01730) by centrifuging at 10^3×g for 15 min (*16*). An internal standard (25 μl methionine sulfone) was added to 75 μl of the filtered serum and muscle homogenate before derivatization. The precolumn derivatization of free amino acids was accomplished with phenyl isothiocyanate to produce phenylthiocarbarnyl amino acids (Pico-Tag Workstation; Millipore, Bedford, MA 01730) and refiltered through a 0.45-μm syringe filter (Acrodisc CRPTFC, Gelman Sciences, Ann Arbor, MI 48106-1448). Concentrations of 38 individual amino acids were determined in derivatized samples with a HPLC (Water Model 820 system controller and Model 501 pumps; Millipore, Bedford, MA 01730). The following chromatographic conditions were used: Waters Pico-Tag Silica/C18 (300×3.9 mm) column; column temperature of 46 °C; flow rate of 1.0 ml/min with back pressure of 5.5x10^3 psi (3.8×10^2 bar); system sensitivity of 489 mV/s (recorder) and 0.5 absorbance units full scale (Waters Model 484 UV detector, set at 254 nm); sample size of 10 μl; and 87 min run time. Solvent conditions and gradients for separating amino acids were those described by Cohen et al. (*16*). Amino acid concentrations were recorded as micromoles per deciliter for serum and nanomoles per gram fresh mass for muscle tissue.

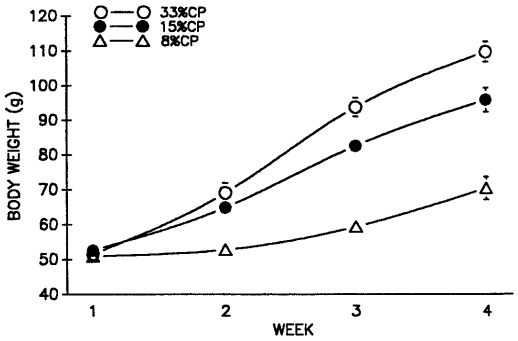

Figure 1. Changes in body masses (x±SE) of juvenile Northern Bobwhites fed formulated isocaloric rations containing either a high (33%), medium (15%), or low (8%) crude protein content.

Statistics. Differences in body condition indices, serum amino acid, and muscle free amino acid concentrations among dietary protein treatments were determined by analysis of variance using the General Linear Models Procedure (*17*). Two quail died during the experiment and were not included in the analyses. An unbalanced data set for serum amino acids resulted from our inability to obtain a sufficient volume of serum from four birds fed the 15% protein diet and one bird fed the 8% protein diet. Differences among means (P<0.05) were isolated by using the least significant difference test. Relationships among serum and muscle free amino acid concentrations were explored using Pearson correlation analysis (PROC CORR; *17*).

RESULTS

Body Condition Indices. Initial body masses averaged 51.8±0.8 g (SE) and did not differ (P=0.680) among treatment groups. Average daily feed consumption for the 33, 15, and 8% protein treatment groups was 11.0, 10.0, and 9.2 g per bird during the trial respectively. Body masses differed significantly (P<0.001) among all three treatment groups at 3 weeks (Fig. 1).

Relative liver mass for the 8% protein group was significantly higher (P=0.001) than the 33 and 15% protein groups (Fig. 2); absolute liver mass did not differ among treatments. Absolute and relative adrenal gland and gonad masses did not differ (P>0.050) among dietary treatments, but absolute mass of the gizzard (P<0.001) was significantly influenced by dietary protein treatment (Fig. 2). Both the 33 and 15% dietary protein groups had absolute gizzard masses greater than chicks in the 8% dietary group; differences between the 33 and 15% dietary protein groups were not significant. Absolute and relative spleen masses were significantly (P=0.037 and P=0.002, respectively) influenced by dietary protein content (Fig. 2). Weights were higher in the 33 and 15% protein group than in the 8% protein group; there were no differences between the 33 and 15% protein groups.

Percentage body fat (P<0.001) was significantly influenced by dietary protein content (Fig. 2). Body fat content was greater among the 33 and 8% protein groups than the 15% group; differences between 33 and 8% protein groups were not significant. Body protein (P=0.002) also was significantly influenced by dietary protein (Fig. 2). Protein comprised a greater percentage of total body dry mass in the 15% protein group than the 8% group. Percentage gizzard fat (P=0.289) was not significantly affected by protein intake.

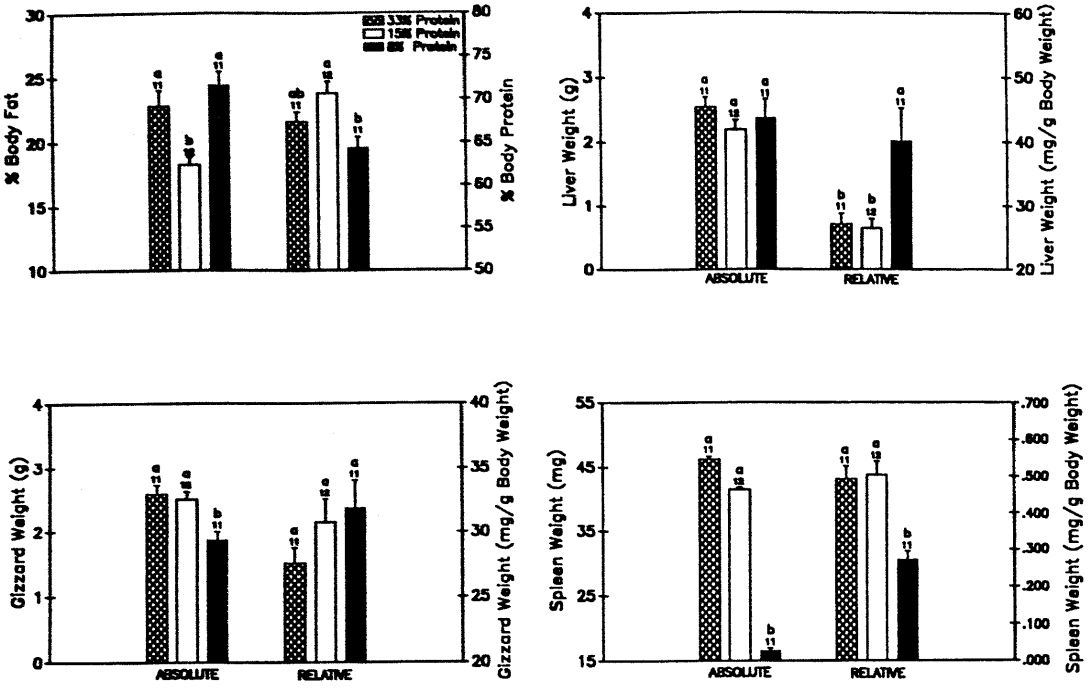

Figure 2. Percentage body fat and body protein, x (±SE) absolute (mg) and relative (mg/g body weight) mass of the liver, gizzard, and spleen in juvenile Northern Bobwhites fed isocaloric diets containing either 8, 15, or 33% crude protein over a 4-week period. Means with different letters are significantly different ($P<0.05$). The number above error bars represent sample size (n).

Serum Amino Acids. Twenty-six amino acids and carnosine, a dipeptide of alanine and histidine, occurred at detectable concentrations (0.46 μmol/dl) in serum (Table 2). Alanine, glycine, serine, and taurine were the most abundant amino acids in serum of Northern Bobwhites. Concentrations of 1-methylhistidine and 3-methylhistidine were low but detected in all individuals.

Total concentrations of neutral amino acids (NAA = leucine + isoleucine + tyrosine + phenylalanine + tryptophan) and branched-chain amino acids (BCAA = leucine + isoleucine + valine) were significantly ($P<0.001$ and $P<0.001$, respectively) lower in 8% than 15 and 33% protein-fed chicks (Fig. 3). Concentrations of SAA (cysteine + methionine) in serum decreased ($P=0.002$) as protein intake increased. Concentration of aromatic amino acids (AROM = phenylalanine + tyrosine + tryptophan) were not significantly different ($P=0.077$) among diet groups. Total concentration of essential amino acids (EAA = arginine + threonine + valine + isoleucine + leucine + methionine + phenylalanine + lysine + histidine + tryptophan) decreased ($P=0.012$) in the 8% protein group in comparison to other diet groups. Concentrations of nonessential amino acids (NEAA = aspartic acid + serine + asparagine + glutamic acid + proline + glycine + alanine + tyrosine) were elevated ($P<0.001$) among chicks fed 8 or 15% protein compared to chicks fed 33% protein. As a result, the EAA:NEAA ratio, as calculated by Gustafson et al. (*11*), was significantly influenced ($P<0.001$) by diet; values increased with increased protein in the diet.

With the exception of arginine, histidine, and methionine, concentrations of individual EAA in serum of chicks were greater for the 15 and 33% dietary protein group compared with the 8% group (Table 2). Isoleucine, leucine, lysine, and valine concentrations were more than twofold greater in the 33% protein group than in the 8% group. Histidine concentration was significantly higher in the 15% protein group than in the 8% protein group.

Individual NEAA were less responsive to dietary protein intake than the EAA (Table 2). Alanine, aspartic acid, cysteine, phosphoserine, serine, and taurine concentrations were greater in the 8% protein

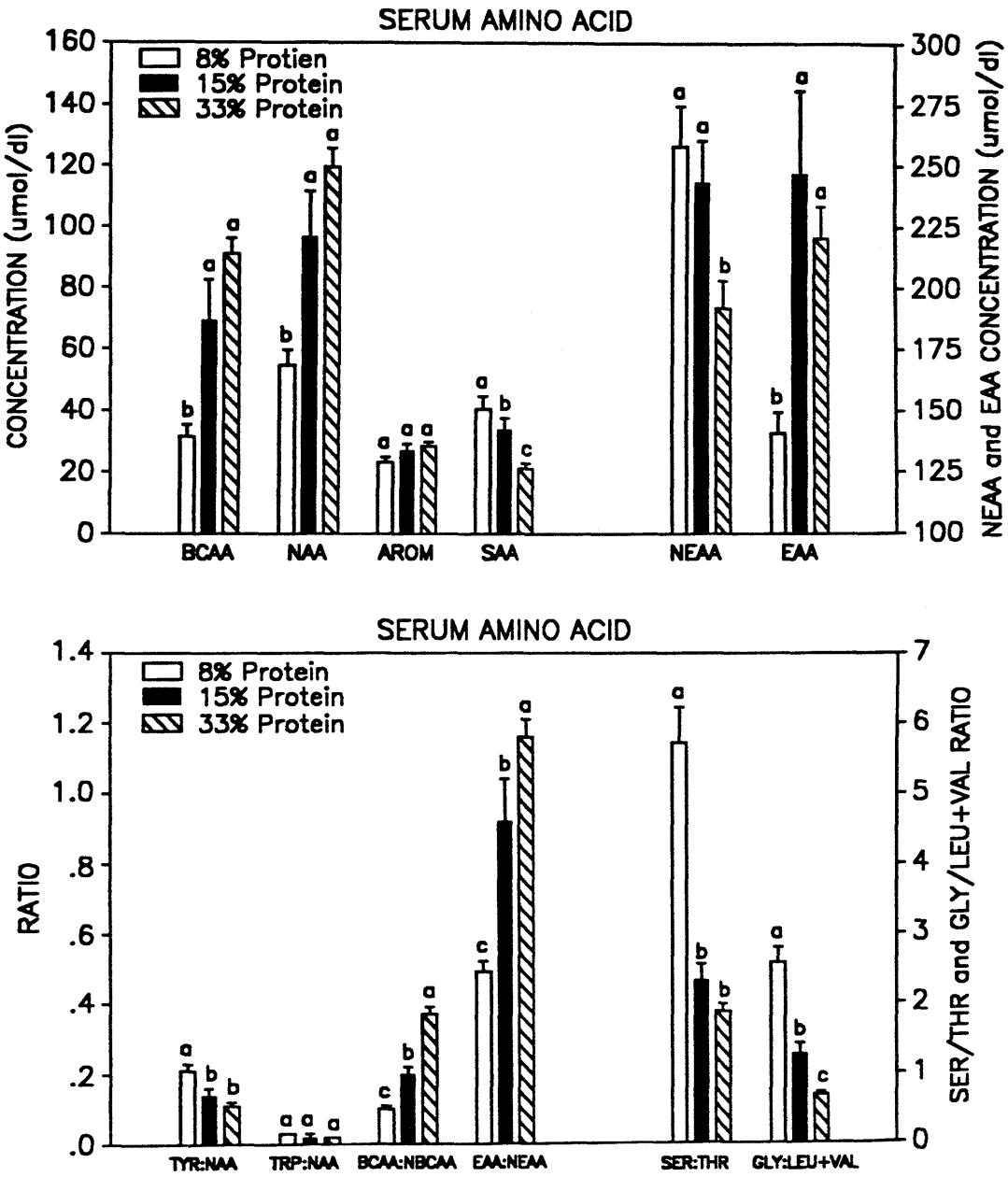

Figure 3. Dietary protein level (8, 15, or 33% crude protein) related to selected free amino acid concentrations and amino acid ratios [BCAA = branched-chain amino acids, NAA = neutral amino acids, AROM = aromatic amino acids, SAA = sulfur-containing amino acids, NEAA = nonessential amino acids, EAA = essential amino acids, TYR:NAA = tyrosine/neutral amino acids, TRP:NAA = tryptophan/neutral amino acids, BCAA:NBCAA = branched-chain/non-branched chain amino acids, EAA:NEAA = essential/nonessential amino acids, SER:THR = serine/threonine, GLY:LEU+VAL = glycine/(leucine + valine)] in serum of juvenile Northern Bobwhites. Means with different letters are significantly different ($P<0.05$). The number above error bars represent sample size (n).

group than the 33% protein group; concentrations of all except aspartic acid were greater in the 15% than the 33% protein group and aspartic acid and cysteine were greater in the 8% than the 15% group.

Ratios of tyrosine:NAA and serine: threonine were significantly lower ($P<0.001$) in chicks fed a 33% protein diet compared with those on 8% protein (Fig. 3). The ratio of glycine:(leucine + valine) differed signifi-

TABLE 2. Mean (±SE) concentrations (μmol/dl) of serum free essential and nonessential amino acids in juvenile Northern Bobwhites fed a diet with 8, 15, or 33% crude protein (CP).

Amino acid	8% CP (n=10)	15% CP (n=8)	33% CP (n=11)
Essential			
Arginine	35.2±2.1	45.7±5.5	42.3±1.7
Histidine	5.65±0.57^{b}	9.10±1.09^{a}	7.08±0.55^{ab}
Isoleucine	6.46±0.96^{c}	16.21±3.43^{b}	23.48±1.44^{a}
Leucine	10.5±1.3^{b}	20.8±3.6^{a}	22.9±1.3^{a}
Lysine	13.6±1.7^{b}	39.5±9.5^{a}	31.7±5.0^{a}
Methionine	17.5±3.0	16.7±2.3	10.3±0.8
Phenyl-alanine	10.3±0.7^{b}	12.8±1.4^{a}	13.3±0.7^{a}
Threonine	12.6±0.9^{b}	27.3±4.6^{a}	22.5±1.5^{a}
Tryptophan	1.52±0.14^{b}	2.01±0.30^{a}	2.37±0.27^{a}
Valine	14.5±1.8^{c}	32.4±6.0^{b}	44.6±2.7^{a}
Nonessential			
Alanine	52.6±5.3^{a}	43.4±4.6^{a}	29.1±2.2^{b}
Asparagine	16.8±1.4	22.6±1.8	17.9±1.6
Aspartic acid	2.36±0.46^{a}	1.23±0.20^{b}	1.14±0.17^{b}
Carnosine	1.41±0.23	1.77±0.19	1.95±0.32
Citrulline	3.43±0.58	3.79±1.00	2.20±0.57
Cysteine	23.0±2.0^{a}	17.1±1.9^{b}	10.8±0.57^{c}
Glutamic acid	19.8±2.2	21.2±1.9	21.6±1.8
Glycine	61.4±6.1	54.3±3.2	46.1±2.8
Hydroxy-proline	6.23±1.11	8.65±1.12	7.95±1.24
1-Methyl-histidine	1.99±0.52	2.00±0.23	1.74±0.20
3-Methyl-histidine	3.33±0.52	3.94±0.62	3.52±0.60
Ornithine	4.19±0.42	7.79±2.01	4.75±0.56
Phospho-serine	6.98±0.92^{a}	6.66±1.05^{a}	2.66±0.35^{b}
Proline	24.1±1.9	28.8±3.5	21.8±2.3
Serine	69.6±5.7^{a}	59.7±4.2^{a}	41.4±2.4^{b}
Taurine	71.1±6.3^{a}	61.6±8.1^{a}	42.2±3.9^{b}
Tyrosine	11.4±1.1	12.3±0.77	12.8±0.93

a,b,c Means in a row with no common superscript differ significantly (P≤0.05).

TABLE 3. Mean (±SE) concentrations (nmol/g) of essential and nonessential free amino acids in fresh muscle of juvenile Northern Bobwhites fed a diet with 8, 15, or 33% crude protein (CP).

Amino acid	8% CP (n=10)	15% CP (n=8)	33% CP (n=11)
Essential			
Arginine	679±65	875±60	803±64
Histidine	316±41^{b}	461±35^{a}	399±33^{ab}
Isoleucine	380±31^{b}	570±36^{a}	486±35^{ab}
Leucine	683±70	776±47	623±44
Lysine	216±23^{b}	311±19^{a}	235±20^{b}
Methionine	419±41^{a}	447±24^{a}	317±27^{b}
Phenyl-alanine	344±31	418±28	352±20
Threonine	498±42^{b}	760±60^{a}	594±53^{b}
Tryptophan	98±7^{b}	129±7^{a}	126±10^{a}
Valine	596±44^{b}	834±59^{a}	749±58^{ab}
Nonessential			
Alanine	1,247±88	1,303±82	1,023±81
Asparagine	707±46^{b}	911±54^{a}	716±66^{b}
Aspartic acid	366±26^{b}	511±36^{a}	458±40^{ab}
Carnosine	4,320±752^{b}	1,0281±810^{a}	1,2935±1072^{a}
Citrulline	48±14	49±8	45±6
Cysteine	393±28^{a}	275±27^{b}	273±9^{b}
Glutamic acid	1,521±199	1,854±82	1,804±187
Glycine	2,133±210^{a}	1,534±93^{b}	1,024±93^{c}
Hydroxy-proline	64±10	53±8	51±9
Ornithine	42±4	39±4	35±11
Phospho-serine	854±201	901±135	700±106
Proline	484±49	497±37	511±40
Serine	1,584±92^{a}	1,428±71^{a}	1,127±75^{b}
Taurine	2,216±239^{a}	1,062±101^{b}	741±50^{b}
Tyrosine	418±37	509±36	411±34

a,b,c In a given row, Means with no common superscript differ significantly (P≤0.05).

cantly (P<0.001) among all diet groups, increasing with decreased protein intake. The BCAA:NBCAA ratio differed significantly (P<0.001) among all diet groups as well, decreasing with decreased protein intake. No diet differences for ammonia (overall x=4.84 μmol/dl; P=0.348) or urea (overall x=58.64 μmol/dl; P=0.123) were detected.

Muscle Free Amino Acids. Twenty-four amino acids and carnosine occurred at detectable concentrations (19.81 nmol/g) in muscle tissue (Table 3). Alanine, glutamic acid, glycine, serine, and taurine were the most abundant free amino acids in muscle tissue of Northern Bobwhites. Carnosine accounted for a large proportion of the measurable free amino acid pool in muscle. 1-Methylhistidine and 3-methylhistidine were not detected in muscle homogenates of experimental subjects.

In general, the total free amino acid pool in muscle tissue tended to be greatest in Bobwhite chicks fed a 15% protein diet. Concentrations of NAA (P=0.030) and BCAA (P=0.023) in muscle tissue were significantly lower in the 8% protein group compared with those fed 15% protein (Fig. 4); concentrations did not differ between the 8 and 33% groups. Concentrations of AROM did not differ (P=0.076) among treatments but the SAA were elevated (P=0.001) in chicks fed the 8% protein diet. In contrast, total concentration of EAA in muscle tissue (P=0.034) was depressed among those fed the 8% protein diet;

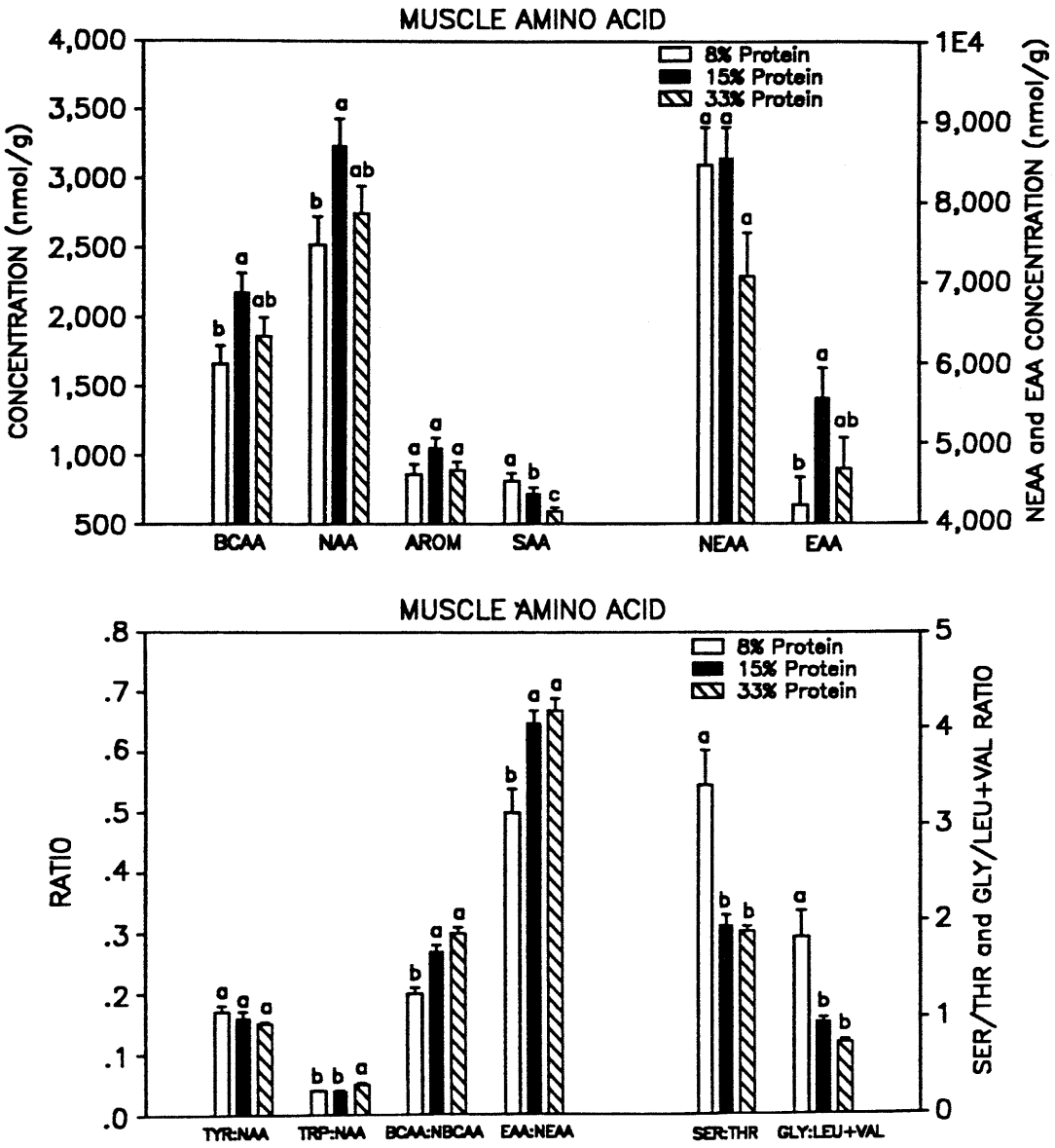

Figure 4. Dietary protein level (8, 15, or 33% crude protein) related to selected free amino acid concentrations and amino acid ratios [BCAA = branched-chain amino acids, NAA = neutral amino acids, AROM = aromatic amino acids, SAA = sulfur-containing amino acids, NEAA = nonessential amino acids, EAA = essential amino acids, TYR:NAA = tyrosine/neutral amino acids, TRP:NAA = tryptophan/neutral amino acids, BCAA:NBCAA = branched-chain/non-branched chain amino acids, EAA:NEAA = essential/non-essential amino acids, SER:THR = serine/threonine, GLY:LEU+VAL = glycine/(leucine + valine)] in breast muscle of juvenile Northern Bobwhites. Means with different letters are significantly different ($P<0.05$). The number above error bars represent sample size (n).

differences in total concentration of NEAA were not statistically significant (P=0.078). The EAA:NEAA ratio was significantly ($P< 0.001$) greater in chicks fed the 15 and 33% protein diets compared with those on the 8% protein diet.

Concentrations of individual EAA in muscle tissue tended to increase in response to moderate (15% protein) protein restriction, but returned to normal (33% protein) or slightly decreased with severe (8% protein) protein restriction (Table 3). Lysine, methionine, and threonine concentrations were greater ($P<0.05$) in the 15% protein group than in the 33% group. Histidine, isoleucine, lysine, threonine, tryptophan, and

valine concentrations were greater ($P<0.05$) in the 15% protein group than in the 8% group; tryptophan was the only EAA that was significantly lower ($P=0.042$) among those fed 8% compared with 33% protein in the diet. Methionine was the only EAA that was significantly greater in the 8% diet group compared with those fed 33% protein.

Among the NEAA carnosine concentrations were nearly three-fold greater in the 33% group compared to the 8% protein group ($P<0.001$; Table 3). In comparison, concentrations of cysteine, glycine, serine, and taurine were significantly greater ($P<0.002$) among chicks fed 8% rather than 33% protein. Asparagine, glycine, and serine concentrations were also elevated in the 15% protein group compared with those fed 33% protein. Taurine and glycine concentrations changed in proportion to dietary protein intake.

The ratios of serine:threonine ($P<0.001$) and glycine:(leucine + valine) ($P<0.001$) in the muscle were greater in chicks fed 8% protein. The tryptophan:NAA ratio was depressed ($P=0.045$) in chicks fed either 8 or 15% protein (Fig. 4). The BCAA:NBCAA ratio of chicks fed 8% protein was significantly lower ($P<0.001$). There was no significant ($P=0.140$) difference among treatments for the tyrosine:NAA ratio.

Ammonia concentrations in muscle homogenates were significantly ($P=0.036$) elevated in the 8 (1,706±188 nmol/g) and 33% (1,757± 187 nmol/g) protein groups compared with the 15% (1,106±121 nmol/g) protein group. Urea concentration was significantly ($P=0.001$) elevated in the 8% (3,351±308 nmol/g) protein group compared with the 15 (2,171±178 nmol/g) and 33% (2,252±122 nmol/g) protein groups.

DISCUSSION

Body Condition Indices. Northern Bobwhites require a 27% crude protein diet for maximum growth and survival to 6-wk posthatch (*18*). The inability of young Bobwhites to acquire sufficient levels of dietary protein results in depressed growth and development as indicated by reduced body masses of juvenile Bobwhites fed either 15 or 8% protein diets in this study.

Metabolically active organs such as the liver will often change in size with altered nutritional states, reflecting physiological adaptation to changes in circulating nutrient levels. Liver mass responds to different physiological states in ring-necked pheasants *(Phasianus colchicus; 19)* and spruce grouse *(Dendragapus canadensis; 20)* and may be a useful measure of condition in Bobwhite chicks. Juvenile Bobwhites showed increased relative liver masses with decreases in dietary protein. However, absolute liver masses were similar, indicating that the increase in relative liver mass was not pathological. Poultry often show similar responses due to increased lipogenesis and fat deposition associated with excessive caloric consumption while trying to meet protein requirements (*21*). Observed changes in body fat of juvenile Bobwhites supports a similar interpretation for liver mass differences among diet groups. Chicks fed low-protein diets may consume an excess of energy relative to their needs as they attempt to meet protein requirements. Auckaland and Morris (*22*) observed similar elevations in body fat of domestic turkey *(Meleagris gallopavo)* poults fed protein-restricted diets. These findings suggest that some caution must be exercised in using body fat as a measure of overall condition in wild Bobwhites (*2*).

Masses of the gizzard and spleen were also useful morphometric discriminators for evaluating body condition in juvenile Bobwhites. The gizzard may serve as a protein reserve source in birds subjected to a dietary protein deficiency through catabolism of muscle protein (*23*). DuBowy (*24*) observed increases in gizzard mass of blue-winged teal (*Anas discors*) in response to a shift in food quality. Atrophy of major lymphoid organs such as the spleen has been demonstrated in mammals subjected to protein malnutrition during early development (*25*).

Free Amino Acids. In general, concentrations of individual amino acids in serum were not linearly correlated with muscle concentrations. Exceptions included serine ($r=0.67$), threonine ($r=0.51$), alanine ($r=0.52$), tyrosine ($r=0.52$), valine ($r=0.48$), and methionine ($r=0.67$), which were significantly ($P<0.01$) correlated between serum and muscle pools.

Alterations in amino acid nutrition as a result of consuming diets low in protein

results in acute changes in rate of amino acid oxidation due to modified enzyme activity and substrate availability, decline in protein synthesis, and altered rates of protein catabolism (*10*). Such physiological adjustments may be important in conservation of important EAA and nitrogen when intake is restricted.

In mammals, consumption of low-protein diets usually initiates a greater depression in concentrations of the EAA relative to the NEAA pool in blood serum, making the EAA:NEAA ratio a useful index for assessing protein nutritional status in humans (*8*). The decrease in EAA and concomitant increase in NEAA observed in the current study may be partly explained by the birds' inability to synthesize EAA but ability to synthesize NEAA as they are utilized (*26*). Essential amino acids may be selectively shunted out of the blood into muscle pools (*27*). The EAA:NEAA ratio also appears to be a useful index for assessing protein malnutrition in quail chicks; the index increased with increasing protein intake. Shunting of EAA from the serum pool into muscle was indicated by depressed serum concentrations (7 of the 10 EAA measured were significantly depressed in serum of chicks fed the 8% protein diet) relative to muscle (elevated or normal concentrations for all EAA except tryptophan in 15 and 8% protein groups). Differences in the EAA:NEAA ratio among diet groups were less apparent in muscle tissue. The glycine:(leucine + valine) ratio is frequently used as an abbreviated index of the NEAA (glycine):EAA (leucine + valine) ratio in human diagnostic medicine (*4*). The glycine:(leucine + valine) ratio in serum and muscle of Bobwhite chicks in response to protein intake was an inverse reflection of the EAA:NEAA ratio.

Because BCAA generally accumulate in serum proportionally with dietary concentration, their concentration or the BCAA:NBCAA ratio has been useful in assessing protein nutritional status in humans and laboratory rodents (*28,29*). The BCAA are important mediators of muscle protein synthesis and are actively taken up by muscle (*30*). Concentrations of BCAA in both muscle tissue homogenates and serum strongly reflected dietary protein levels in juvenile Bobwhites. Depressed concentrations of serum and muscle BCAA among quail on a low-protein diets were due largely to reductions in concentrations of isoleucine and valine, which is in agreement with observations in protein-malnourished mammals (*29*). The BCAA are catabolized by oxidation in the muscle and serve as key nitrogen sources for the transamination of pyruvate to alanine, resulting in a net increase in serum alanine concentration (*31*), as was observed among chicks fed low-protein diets.

Concentrations of SAA in both serum and muscle pools were elevated among Bobwhites subjected to a low-protein diet due to increases in both cysteine and metbionine. Elevations in the SAA especially cysteine, of Bobwhite chicks during protein restriction may have been related to decreased mobilization of cysteine for feather development, which occurs rapidly at this age (*32*). Taurine, a nonprotein amino acid, is a product of cysteine catabolism in birds and is often elevated in protein-malnourished animals (*33*). Muscle and serum taurine concentrations were greatly elevated in protein-restricted Bobwhites (200% increase over muscle concentrations of controls fed high levels of protein), which may reflect an increase in cysteine catabolism to taurine rather than pyruvate as a result of the dietary protein deficiency.

Elevated concentrations of alanine, serine, and phosphoserine in serum and glycine and serine in muscle homogenates of Bobwhite chicks fed in low-protein diets agree favorably with studies by Lunn et al. (*27*) with protein-restricted laboratory rats. Elevated serine concentrations may have been related to the elevated concentrations of glycine, which can be converted to serine via serine hydroxymethyl transferase. The large overall increases in concentrations of alanine, glycine, serine, and other NEAA in protein-malnourished Bobwhites indicates a general adaptation to the high-caloric and low-protein diets by increasing synthesis of NEAA, possibly as a nitrogen-conserving strategy. Antener et al. (*31*) found the serine:threonine ratio to be a useful index for assessing protein malnutrition in human patients and was a sensitive index for Bobwhite chicks in this study.

The reduction in carnosine concentration in the free amino acid profile of muscle tissue of Bobwhite chicks fed protein-

restricted rations was one of the most notable alterations. Carnosine, a dipeptide of histidine, occurs at high concentrations in skeletal muscle and acts as a buffer to lactic acid accumulation when muscle tissue undergoes metabolism, particularly during exercise (*34*). Nutritionally induced suppression of carnosine in Bobwhites could be a response to overall declines in metabolic activity.

Recommended Indices of Condition. The present results support the continued use of traditional morphometric indices such as masses of carcass and selected metabolically active organs to assess the nutritional status of developing Northern Bobwhites. Measures of fat stores are also useful for assessing chronic caloric intake, but do not necessarily provide a useful index of protein intake.

Free amino acid pools of both blood serum and muscle tissue are dynamic and respond to acute alterations in dietary protein. In general, Bobwhite chicks fed a low-protein diet responded in a fashion that was remarkably similar to previously documented cases of protein malnutrition involving humans and laboratory rodents. Several of the diagnostic indices used in clinical nutrition assessment in human medicine appear equally suited for use in Bobwhites. Total concentrations of SAA and ratios of BCAA:NBCAA, EAA:NEAA, and glycine:(leucine + valine) in blood serum were excellent discriminators among diet groups. Likewise, concentrations of SAA and glycine in muscle tissue homogenates were useful discriminators among diet groups.

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